Journal of the Lepidopterists' Society
37(2), 1983, 160–163

A BRIEF DESCRIPTION OF THE PHYSIOLOGICAL TECHNIQUES—DISC GEL ELECTROPHORESIS, INCLUDING GEL PHOTOGRAPHY AND THIN LAYER CHROMATOGRAPHY

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ABSTRACT. Two physiological techniques are described: disc gel electrophoresis, including photography of the electrophoresis gels, and thin layer chromatography. Modifications of the basic techniques have been worked out which give the best results with antennal esterases from the cabbage looper moth, *Trichoplusia ni* (Hübner).

The following physiological techniques were developed during studies for the M.S. Degree in Entomology at the University of Florida, Gainesville. The goal of the research was to study the esterases, during late pupal and adult development, in the antennae of the cabbage looper moth, *Trichoplusia ni* (Hübner) and the role these enzymes may play in pheromone breakdown. Dr. Lee Miller of the Allyn Museum of Entomology, Sarasota, Florida, advised me these techniques may also be of interest to taxonomists. Discussed are the following methods: disc gel electrophoresis, photography of the gels, and thin layer chromatography.

Disc Gel Electrophoresis

The basic procedure used was that of Davis (1964). Several additions to, or modifications of, this technique were developed: 1, a Sage Instrument Syringe Pump, Model 355, was used to layer distilled water onto the tops of gels before polymerization so that they would have a flat top; 2, the separating gel (1.5 ml) was placed in gel tubes of 110 mm length and 4.5 mm internal diameter to give better resolution; 3, all solutions were made fresh after 2 weeks, and the ammonium persulfate and solution F were made fresh weekly; 4, two milliamps of current per gel tube were used initially in the studies, but it was later found that 3–4 mamps per tube produced more distinct bands; 5, 0.5 ml of 0.001% bromophenol blue, which served as a front marker, was added to 50 ml in the upper chamber of Tris-glycine buffer, pH 8.3; 6, microsyringes and micropipettes were used to layer samples in sucrose through the buffer in the upper chamber onto the tops of the stacking gels.

The electrophoresis run was terminated when the bromophenol blue front marker was about 5 mm from the bottom of the gel tube, and staining for esterases was carried out immediately. The cabbage

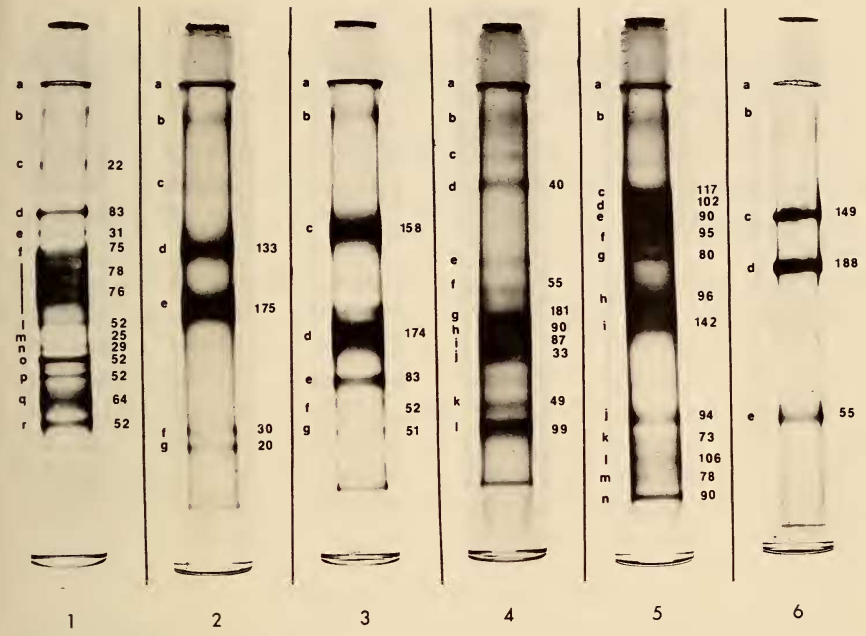

FIG. 1. Disc gel electrophoretic comparison of esterases from the antennae of female cabbage looper moths with females of five other species of moths (USDA, Gainesville, Florida colonies): **1,** cabbage looper, *Trichoplusia ni* (Hübner); **2,** fall armyworm, *Spodoptera frugiperda* (J. E. Smith); **3,** beet armyworm, *Spodoptera exigua* (Hübner); **4,** corn earworm, *Heliothis zea* (Boddie); **5,** velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner); **6,** Indian meal moth, *Plodia interpunctella* (Hübner). Letters indicate bands, while numbers refer to densitometric absorbance, as measured in mm from scan.

looper (*Trichoplusia ni*) antennal esterases were detected using α-naphthyl acetate and Fast Blue RR Salt, according to the procedure of Turunen & Chippendale (1977) with the following modifications: 1, inhibitors were added to enzyme samples before electrophoresis, rather than to the gels following; 2, one ml of acetone was used to dissolve the α-naphthyl acetate before addition of phosphate buffer; 3, the amount of Fast Blue was 3 or 4 mg less than twice the amount of α-naphthyl acetate; 4, the phosphate buffer had a pH of 6.8; 5, following electrophoresis, the gels were not put into a borate solution, but rather were placed directly into α-naphthyl acetate and Fast Blue stain; 6, after staining was completed, the gels were stored in the following fixative (Mauer, 1971): methanol : water : glacial acetic acid (45:45:10). The esterase stain should be made fresh immediately before use.

Gel Photography

The gels were photographed as soon as they were stained with the α-naphthyl acetate and Fast Blue. The gels were left in small glass tubes with fixative and were positioned on a light box covered by a piece of ¼″ thick opal glass. A circular 22 watt fluorescent light was placed 10 cm below the glass. The insides of the box were covered with aluminum foil, and the bottom inside was covered with white paper.

A Nikon F-2 camera with a 55 mm Micro Nikor lens was used to photograph the gels using Kodak estar base, black and white, SO-115 (now Technical Pan Film, #2415), shot at $^1/_8$ second at f11. The film was developed at 20°C (68°F) with D-76 : water (1:1) for seven minutes. Prints were made on fresh Polycontrast or Kodabrome II, medium, glossy paper, and were developed in Dektol : water (1:2). Fig. 1 demonstrates the results of these techniques with a comparison of antennal esterases from the female cabbage looper moth, *T. ni*, and five other species of female moths.

Thin Layer Chromatography

The cabbage looper pheromone, (Z)-7-dodecene-1-ol acetate, is hydrolyzed into the following products: (Z)-7-dodecene-1-ol + acetic acid. The acetate moiety was tritiated. The two products could be separated by the following method. Gelman ITLC-SA, 20 cm × 10 cm thin layer paper was cut into pieces 2½ cm × 10 cm and ovendried at 100°C for 15–20 minutes. Two hundred and fifty ml beakers were used as developing chambers, which were covered with glass Petri dishes. Seven ml of developing solvent, composed of 15 parts of ethyl acetate and 85 parts benzene, were placed in the bottom of the beaker. Absorbant paper (Whatman chromatography) was placed against the inside wall of the beaker and immersed in the solvent to maintain a saturated atmosphere. Ten microliter reaction samples were spotted 1 cm from the bottom of the plate, and the plates were developed for 10–15 minutes or until the solvent front was about 1 cm from the top of the plate.

The tritiated acetic acid product stayed at the origin 1 cm from the bottom of the plate, while the unreacted pheromone went with the solvent front. The alcohol product was unlabeled and not measured by this system, although it was verified that the alcohol went up the plate to a point between the pheromone and the acetic acid at the origin. After development, the plate was cut into sections containing the labeled acetic acid and labeled pheromone and counted, using Instagel (Packard) in a Packard scintillation counter.

ACKNOWLEDGMENTS

I thank Dr. S. M. Ferkovich, USDA, ARS, Gainesville, Florida, in whose laboratory this research was performed, for valuable advice and numerous discussions.

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